TECHNICAL REPORT
NATICK/TR-05/007

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ANTIFUNGAL ACTIVITY OF VOLATILE OIL OF MUSTARD (VOM)

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March 2005

Final Report
January – March 1997

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REPORT DOCUMENTATION PAGE

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1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
02-03-2005	Final	January - March 1997

4. TITLE AND SUBTITLE
ANTIFUNGAL ACTIVITY OF VOLATILE OIL OF MUSTARD (VOM)

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER
D650

6. AUTHOR(S)
A. Sikes,* T. Yang,* M. Richardson,* and R. Ehioba**

5d. PROJECT NUMBER
DA 1Y665709D650

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
*U.S. Army Research,Development andEngineering Command,
Natick Soldier Center, Combat Feeding Program, Natick, MA 01760-5018

**Campbell Soup Co., Campbell Place, Camden, NJ 08103

8. PERFORMING ORGANIZATION REPORT NUMBER
NATICK/TR-05/007

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Volatile oil of mustard (VOM)) is a volatile extract of mustard seed oil that has demonstrated antifungal and antibacterial activity in food items. The active volatile antimicrobial factor in VOM is allyl isothiocyanate (AIT). To evaluate the efficacy of VOM as a fungistatic agent, military-type sandwiches and commercial cheddar cheese samples were inoculated with several mold isolates (spot inoculated with ~10^6 spores/ml) and a yeast/mold cocktail (~$10^{4.5}$ spores/g), respectively. In the absence of VOM, with or without potassium sorbate (Ks), mold growth in the sandwiches occurred between 5 and 10 days at 25°C. However, at vapor concentrations of 300-500 ppm, VOM inhibited growth of several mold species on shelf-stable sandwiches for more than 100 days at 25°C. Yeast and mold growth in cheese samples packaged in meal, ready-to-eat (MRE) pouches were inhibited by 15-25 ppm VOM, but not when cheese samples were packaged in O_2-permeabile polyester film. This study demonstrated that VOM could be an effective fungistat in foods (e.g., sandwiches and cheese products), if an effective concentration was maintained over the food item.

15. SUBJECT TERMS

ANTFUNGAL EFFECTS	BACTERIA	FUNGISTAT	MILITARY RATIONS
ANTIBACTERIAL EFFECTS	YEASTS	CHEESES	MICROBIOLOGY
ANTIMICROBIAL AGENTS	MOLD	SANDWICHES	FOOD PRESERVATION
ALLYL ISOTHIOCYANATE	SHELF LIFE	VOM (VOLATILE OIL OF MUSTARD)	

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Anthony Sikes/Tom Yang
U	U	U	U	35	19b. TELEPHONE NUMBER *(Include area code)* (508) 233-4383/233-4916

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PREFACE

This study was conducted from January to March 1997 by Mssrs. Anthony Sikes and Tom Yang, and Ms. Michelle Richardson of Combat Feeding Directorate, Natick Soldier Center, Natick, MA, and Mr. Robson Ehioba of Campbell Soup Co., Camden, NJ. The work was funded under the D650 program (DA 1Y665709D650) " Slow Release WasaOuro (WO) or VOM Products-Antibacterial/Antifungal Agents" and titled "Antifungal activity of mustard oil extract". It was also supported under a Cooperative Research and Development Agreement (CRADA) between manufacturer of VOM, Carex Corporation, and Natick.

The purpose of the research was to validate the strong antifungal effect of VOM against common mold and yeast that affect the safety and quality of certain military bakery items. A challenge study was conducted on Natick-developed, shelf stable, ready-to-eat sandwiches and commercial cheddar cheese samples; especially when they were stored at ambient temperature with relatively high humidity.

ANTIFUNGAL ACTIVITY OF VOLATILE OIL OF MUSTARD (VOM)

INTRODUCTION

The antimicrobial properties of essential oils of plants of the family *Cruciferia* (genus *Brassica)*, e.g., mustard seeds, have been known for many years (Walker et al., 1925; Block, 1985). The volatile extract of mustard seed oil (VOM), allyl isothiocyanate (AIT), has been shown to be an effective antimicrobial agent against fungi and bacteria, with fungi (yeast and molds) being more sensitive than bacteria. Among bacteria, gram-positive organisms are more sensitive than gram-negative (Isshiki et al., 1992; Sekiyama et al., 1996).

More recently, Lin et al. (2000) reported that the antibacterial effectiveness of two isothiocyanates (ITCs) against several foodborne pathogens was a function of species and exposure time. Worfel et al. (1997) also demonstrated the suppressive effects of mustard seed oil extract against insects that infest grain foods. Previous studies have demonstrated that the main antimicrobial factor in mustard seed oil extract is AIT (Clark, 1992). The mode of action of VOM against yeast, mold and bacteria is not well understood; however, VOM may act by inhibiting proteins or metabolic enzymes (Delaquis and Sholberg, 1997). Respiratory enzymes are especially vulnerable toVOM (Kojima and Ogawa, 1971; Delaquis and Mazza, 1995).

As a food preservative, VOM has been approved for food use in Japan, provided that the compound is extracted from natural sources (Delaquis and Mazza, 1995). Recently, VOM received regulatory clearance (FDA) for use as an antibacterial film for various foods use in North America (Jan 2004). Prior to its current regulatory status, VOM had already been approved in the U.S. for use as a flavoring agent.

The objective of this investigation was to evaluate the antifungal efficacy of a commercial preparation of VOM (WasaOuro^{TM}, Carex Corporation, Osaka, Japan) to control yeast and mold growth on a military ration-type intermediate moisture food products (IMF) and cheddar cheese.

MATERIALS AND METHODS

Preparation of mold inoculum for sandwich study

Mold cultures used in the sandwich inoculation experiment were obtained from the following sources: commercial meat sticks (*Penicillium* sp., designated as MSI), Natick Soldier Center's Laboratory Culture Collection, Natick, MA, (*Aspergillus* sp., designated as M4), and a *Penicillium* sp. (designated as API) was isolated on plate count agar (PCA) from the air of Natick's baking facility. Cultures of the organisms were maintained on plates of potato dextrose agar (PDA; Difco, Detroit, MI) at 4°C.

To prepare spore inocula for the sandwich study, mold cultures were grown on PDA plates at 25°C for 48-72 h (Powers and Berkowitz, 1990). Mold spores were harvested by aseptically adding 5-10 mL of 0.01% (w/w) sterile Tween 80 (Sigma Chemicals, St. Louis, MO) to PDA plates containing mold growth. Spores were dislodged from conidiophores by gently brushing with a sterile inoculating loop. The resulting spore suspensions were filtered through two layers of sterile cheese cloth to remove mycelial debris. Then, each spore suspension was adjusted in sterile 0.05 M phosphate buffer (pH 7.0) to give a final spore density of ca. 10^4 - 10^5 spores/10 µL. The actual numbers of viable spores/mL were determined on PDA plates at 25°C for 48 -72 h.

Preparation of yeast and mold cultures for cheese study

To prepare stock cultures of yeast and molds for the cheese study, a 1:10 slurry of a commercial cheddar cheese product (obtained from local retail outlet) was prepared by blending in sterile deionized H_2O. One ml of the suspension was placed in several sterile petri plates with ca. 20

ml of molten (~ 45°C) potato dextrose agar (PDA) and 40 ppm chlorotetracyline-HCl (Sigma Chemicals, Koburger and Marth, 1984). Plates were incubated at 25°C for 4-5 days.
Two mold isolates were randomly picked (based on color) and purified by repeated streaking on PDA before being identified (microscopically) as *Penicillium* spp. Pure cultures of each isolate were maintained on PDA and stored at 4°C until used.

From the same PDA plate, two yeast colonies were also isolated. Based on microscopic observations and colonial morphology (red-pigmented colonies and budding during vegetative growth), one isolate was identified as *Rhodotorula* sp. and the other was characterized by cream-colored colonies that budded during vegetative growth on PDA. Isolates were picked and streaked on PDA plates and allowed to grow out at 30° C for 24 h. Subsequently, plates were flooded with sterile 0.05 M phosphate buffer (pH 7.0), and the resulting cell suspensions were washed (3x) in sterile 0.05 M phosphate buffer (pH 7.0) and centrifuged (DRP-6000, International Equipment Company, Needham, MA) at 1000 x g for 15 min. Washed cells were resuspended in sterile 0.05 M phosphate buffer (pH 7.0) and stored at 4°C until used. Yeast and mold counts (PDA) were determined on all stock cultures. The average (n = 3) yeast count was
3.8×10^7 cfu/mL and 3.1×10^7 mold spores/mL, respectively.

Preparation of sandwiches

Sandwich bread and components were freshly prepared in Natick's pilot plant. One dough formulation consisted of the following ingredients (% weight): flour (49.68), General Mills, Minneapolis, MN; water (28.96), shortening (8.55) Crisco, Cincinnati, OH; glycerol (6.34), KIC Chemical, Armonk, NY; yeast (2.25), Red Star, Milwaukee, WI; salt (1.29), Morton Intl, Chicago, IL; sucrose ester (1.0), Montello, Inc., Tulsa, OK; glucono-delta-lactone((0.65), Balchem, Slate Hill, NY; gum arabic (0.5) Gum Technology Corp., Tuscan, AZ; calcium sulfate (0.25), ADM

Arkady, Olathe, KS; xanthan gum, (0.04), Kelco Biopolymers, San Diego, CA; encapsulated potassium sorbate (0.1), Balchem, Slate Hill, NY and cream flavor (0.03), Haarmann & Reimer Corp., Springfield, NJ.

The second dough formulation was prepared with all of the above ingredients except potassium sorbate. The meat formulation consisted of the following ingredients (% weight): beef, bottom round (88.039), locally purchased, glycerol (4.0), KIC Chemical, Armonk, NY; water (3.0); salt (2.9), Morton Intl., Chicago, IL; peppercorn (0.5), McCormick & Co., Inc., Hunt Valley, MD; liquid smoke (0.3), Red Arrow Products Co., Manitowoc, WI; black pepper (0.125), McCormick, Hunt Valley, MD; ascorbyl palmitate (0.05), Roche Vitamins, Parsipanny, NJ; lactic acid starter culture (0.031), Diversitech Inc., Alachua, FL; Tenox GT-2 (BHT-0.02), Tenox 4A (BHA-0.02), Eastman Chemical, Kingsport, TN and sodium nitrate (0.015), Dirigo Spice Co, Boston, MA. Beef was ground through a 3/4 inch plate and mixed with other ingredients. The mixture was then ground through a 5/32 inch plate, stuffed into casings (Devro #23A02, Devro-Teepak, Inc.), smoked (Grand Prize^{TM} II, Koch Equipment LLC, Kansas City, MO), and packaged in tri-laminated pouches. The bread and meat were processed according to the procedure outlined in Fig. 1.

Inoculation and storage of meat stick sandwiches

Clear Scotchpak, polyester pouches (heat sealable polyester film, 4.125 x 6.375 inches, O_2 transmission rate: 5 cc/100 sq. in/24 hrs; Water Vapor Permeability: 3.1 gm/m^2/24 hr; Kapak, Corp., Minneapolis, MN) were used to package and store meat stick sandwiches. Septa were prepared by placing a small dollop of silicone (1000 Sealant SCS 1001 Translucent, G.E. Co., Waterford, NY) sealant to the cleaned (alcohol scrubbed) surface of Scotchpak pouches and allowed to dry for 24-48 h (Powers and Berkowitz, 1990).

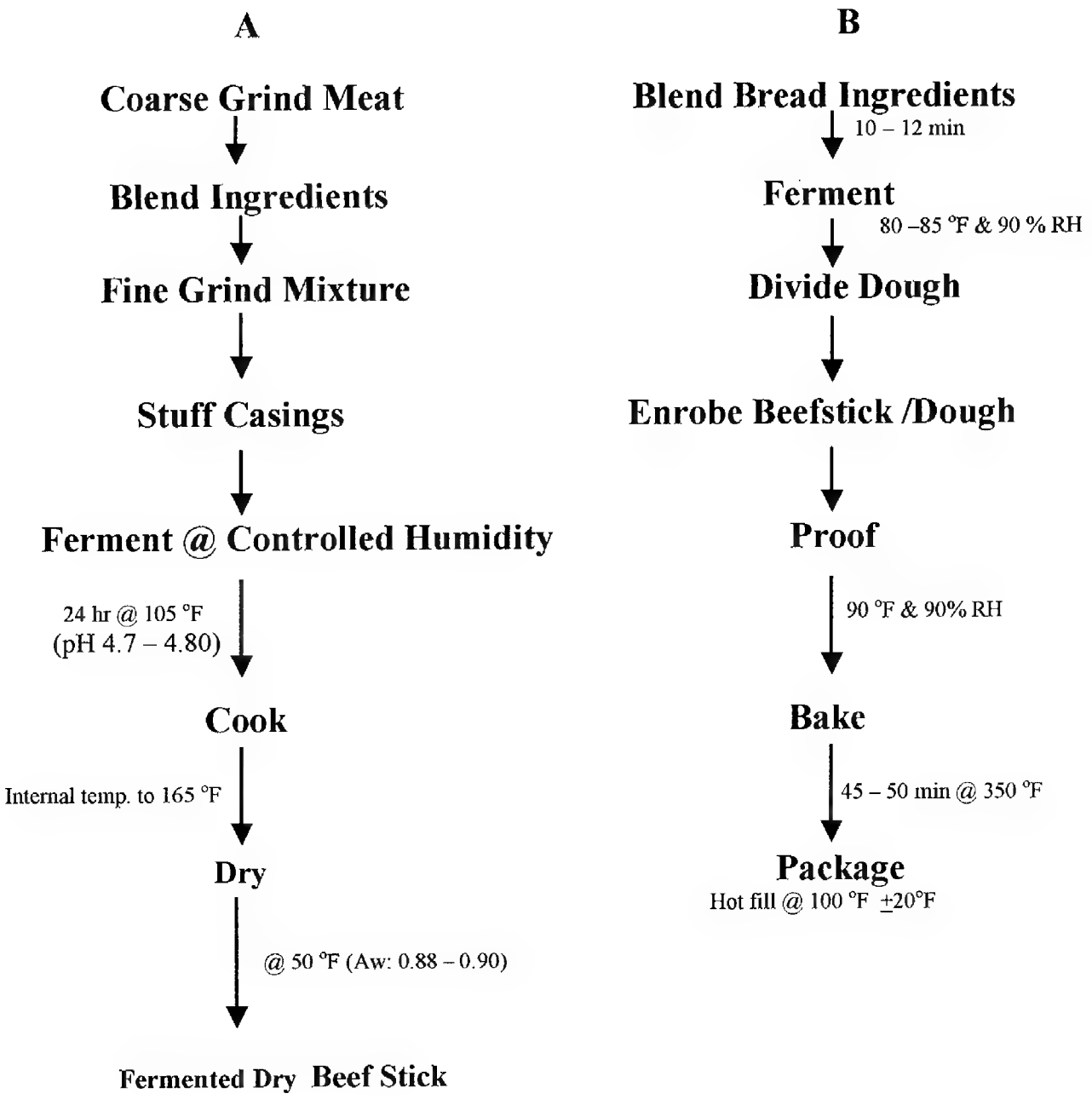


Figure 1. Process flow diagram of beef stick (A) and sandwich (B) production

After baking, breaded enrobed meat sticks (Fig. 1) were cut in half, cooled to 25°C and manually placed in polyester pouches (4.125 x 6.375 inches; Kapak, Corp., Minneapolis, MN). Each pouch, containing 1/2 sandwich, was heat sealed (Pouch Sealer, Kapak, Corp., Minneapolis, MN), and inoculated with *Aspergillus* sp (M4), *Penicillium* sp. (MSI), or *Penicillium* sp. (API). Approximately 10 µL of a spore suspension containing 10^6 conidia/mL was delivered to the bread/meat interface through the self-sealing septa previously placed on the pouches by using a 100 µL sterile syringe (Becton, Dickinson and Company, Rutherford, NJ). After inoculation, 4 VOM labels (Fig. 2a, #LN30D 40 mm x 40 mm) were affixed to each pouch (side opposite the silicone spot) before placing in glass desiccator jars for storage at 25°C.

Storage conditions for enrobed meat sticks

The enrobed beef sticks experiment was divided into two parts. In the first part, each test organism (API, M4, and MSI) was inoculated (in triplicate) at meat/dough interface of 6 breaded beef sticks (cut in half) containing potassium sorbate (Ks) in the dough formulation, and 6 beef sticks containing no Ks. Three sandwiches containing each mold with or without Ks was stored in large, closed desiccator jars (without VOM) at 25°C in the presence of low (50%) or high (94%) relative humidity (RH). A total of 12 desiccator jars was used. In each desiccator, 6 pouches representing 3 experimental conditions, e.g., RH (low or high), VOM (absent), and Ks (absent or present) were stored. The second part of the experiment was setup similar to the first, except VOM labels were placed on each pouch before storage at the two relative humidities (50% and 94%). The high humidity environment inside the desiccator was maintained by using a saturated slurry of potassium nitrate (Kitic et al., 1986; J.T. Baker Chemical Co., Phillipsburg, NJ); while the low humidity environment was the humidity of the ambient air inside the dessiccators. The humidity inside the desiccators was monitored by using a hand held, digital

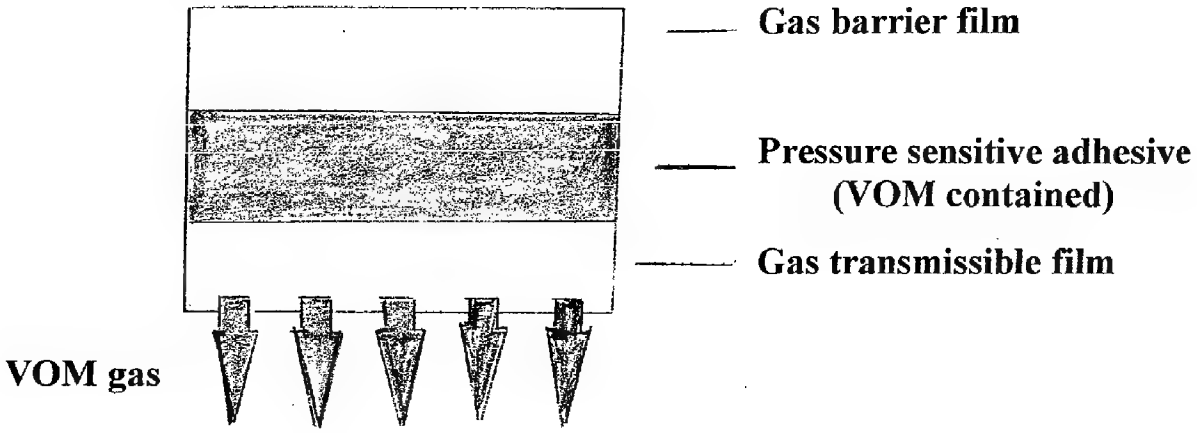
thermohygrometers (model 37101-00, Cole-Parmer Instrument Co., Vernon, Hills, IL). Sandwiches were observed every 5 d (up to 120 d) for visible signs of mold growth.

Preparation and inoculation of cheese samples

Bags (226 g) of shredded cheddar cheese (locally purchased) were aseptically opened and blended with a Hobart mixer (Hobart Mfg. Co., Troy, OH) until well blended. The blended cheese was inoculated with a cocktail consisting of a mixture of two yeast and two mold species previously isolated from commercial cheddar cheese. As determined on PDA plates, the final yeast and mold density/g of product was 103 CFU /g and 103 spores/g, respectively.

Approximately 40g of the inoculated cheese product were aseptically placed in sterile, high-grade, polyethylene stomacher bags (H2O vapor permeability: 1.2 gm/m2/24hr; O2 transmission rate: 12,000 gm/ m2/24 hr; 101mm x 152 mm, Seward Medical, London, England) and heat sealed with a pouch sealer (Kapak, Corp., Minneapolis, MN). The test protocol per pouch of cheese was as follows: Control, inoculated - no VOM labels; T-I, inoculated w/two 40 mm x 40 mm VOM labels; T-II, inoculated w/four 40 mm x 40 mm VOM labels; and T-III, inoculated w/four 40 mm x 55 mm VOM labels (Fig. 2b, #MN10E—packaging mat). For each treatment, 3 bags of product were assigned to each sampling period, e.g., every 7 days. After VOM labels were placed on outside of sealed stomacher bags, they were placed in Scotchpak pouches (heat sealable polyester film, O2 transmission rate: 5 cc/100 sq. in/24 hrs; Water Vapor Permeability: 3.1 gm/m2/24 hr) or MRE pouches, (trilaminated: polyester/ aluminum foil/high density polyethylene) and heat sealed. Approximately 120 cc of air was subsequently delivered through the self-sealing septum (dollop of silicone) to each pouch by using a 60 cc disposable syringe (Sherwood, Deland, FL).

(1).Construction



(2) Application

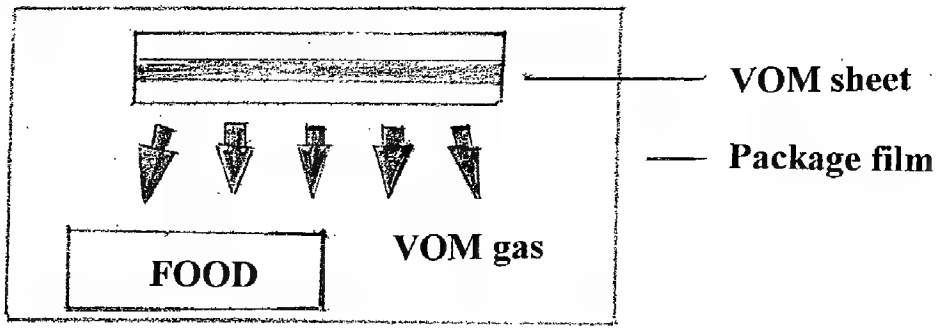


Figure 2a. Diagram of VOM labels used in storage study of military-type ratio sandwiches (#LN30D-inner packaging label)

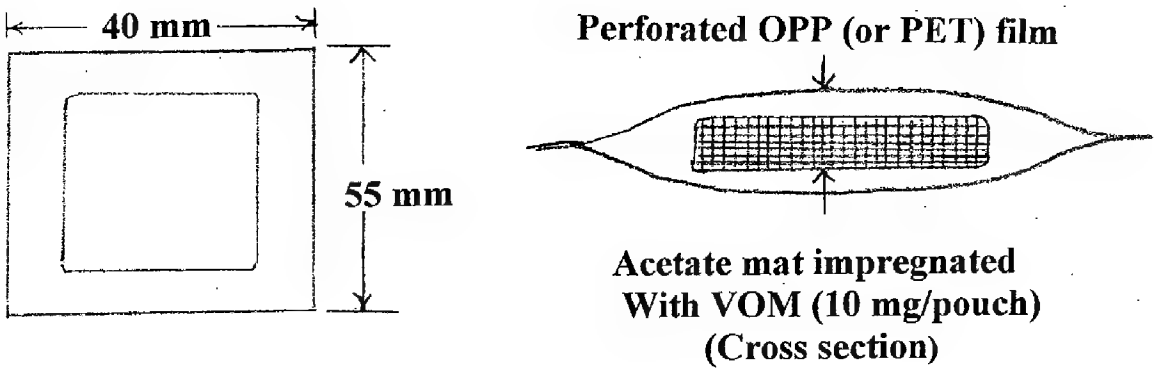


Figure 2b. Diagram of VOM labels used in the storage study of commercial cheddar cheese (#MN10E-packaging mat)

The addition of air facilitated gas (VOM) penetration of the food product, and consequently, yeast and mold growth. All packaged samples were stored in a 25°C incubator and removed for analysis every 7 d.

Yeast and mold counts (YMC) in the cheese samples were determined on potato dextrose agar (PDA) plates by methods similar to those described by Koburger and Marth(1984). To cooled (~45°C) PDA, 40 ppm chlorotetracyline (Difco, Detroit, MI) was added before 15 to 20 ml was dispensed into sterile petri dishes. At the end of each incubation period (48 -72 hr at 25°C), the entire cheese sample (40g) was blended (Stomacher Lab Blender 80, Tekmar Co., Cincinnati, OH) for 2 min. Ten (10g) grams of the blended cheese sample was added to 90 ml of 0.05 M sterile phosphate buffer (pH 7.0) and stomached for 2 min. Serial dilutions were subsequently made, and 0.1 ml aliquots were spread on modified PDA plates and incubated for 48 - 72 h at 25°C. Yeast and mold counts were expressed as YMC/g of product.

Water activity (a_w) and pH measurements

Water activity determinations ("free water") of the enrobed meat sticks and cheddar cheese samples were measured at the beginning and conclusion of the storage studies. A_w values were measured on the Cheddar cheese samples, and the enrobed meat stick. The process involved removing the fermented meat from the bread and determining the a_w of each component: bread, meat, and the combination of both. A minimum of five samples each of the intact sandwich, and components were blended separately at high speed in a sterile Waring blender (Dynamics Corp. of America, New Hartford, CT) for 1 minute. Subsequently, 2-4 g of ground sample of each product (meat, bread and combination) were placed in sample cups, and a_w measurements were performed using a Aqua Lab CX -2 instrument (Decagon Devices, Inc., Pullman, WA).

The reliability of the instrument was checked before and after use against saturated solutions of KCl and NaCl according to the procedures described in Aqua Lab CX -2 owners manual (Decagon Devices, 1994). All a_w values were the mean of 5 samples $\pm$ the standard deviation.

An Orion Expandable Analyzer, Model EA 920 (Orion Research, Boston, MA) was used to measure pH. After grinding the product (cheese, meat, bread, and bread/meat combination), slurries (1:1, w/w) of each product in boiled, distilled water (AOAC, 1990) were prepared, and the pH was determined. The pH of five (5) samples of each ground product was determined at the beginning and conclusion of each study. Final values were the average of 5 replicates.

VOM labels and Measurement of vapor concentration

Two types of commercial VOM labels (WasaOuro^{tm}) were used in the present study: WasaOuro (VOM) sheets (LN30D; 40 mm x 40 mm, Fig 2a), and WasaOuro (VOM) mats/sachet (MN10E; 40 mm x 55 mm, Fig. 2b). The VOM content of the WasaOuro sheets and pouches (sachet) was 3 g/m^2, and 10mg/pouch, respectively. VOM labels were obtained from Carex Inc., Osaka, Japan.

Measurements of VOM levels in closed glass desiccator jars were determined by analyzing the headspace in the desiccators. Sampling was accomplished by fitting the hose connection of the sleeve valve on the desiccator with a sleeve stopper (Fisher). By using an aspirating pump (Model AP-1, Komyo Rikagaku Kogyo K.K., Tokyo, Japan) with a detector tube (Type # 1, Komyo Rikagaku Kogyo K.K., Tokyo, Japan) connected to a 22-gauge syringe needle, AIT gas samples were pulled across the sleeve stopper into the detector tube. VOM vapor readings were obtained directly from the scale printed on the detector tube (Komyo Rikagaku Kogyo K.K., Tokyo, Japan).

For samples stored in polyester film and MRE pouches, VOM gas levels were taken through the self-sealing silicone septum formed on the surface of each pouch, by using the same aspirating pump system described above. Five determinations were made on each packaged sample at each sampling period.

Statistical analysis

Differences in $\log_{10}$ microbiological counts among the cheese sample (polyester film and MRE pouches) treatments were examined for significance by one-way analysis of variance and Duncan's multiple range comparisons (Minitab™ Statistical Software, Minitab Inc., State College, PA).

RESULTS AND DISCUSSION

Control of mold growth on shelf stable sandwiches with gaseous AIT

The ability of allyl isothiocyanate (AIT) to inhibit mold growth on a military-type, enrobed meat stick was evaluated using shelf-stable sandwiches and shredded Cheddar cheese. At 10 d of storage, (25°C) under low RH (50%), 39% of the inoculated sandwiches stored without VOM exhibited visible surface mold growth; however, under similar storage conditions, except for a high RH (94%), 56% of inoculated sandwiches exhibited visible mold growth during the same storage period (Table 1a). Between 10 and 25 d of storage (25°C), all sandwiches stored without VOM exhibited visible mold growth. The relative humidity condition in the storage environments of the inoculated sandwiches appeared to have some limited influence on the rate of mold growth (Table 1b).

The presence of potassium sorbate (antimycotic agent used in the bakery industry) in the bread appeared to delay the onset of visible growth of some of the mold isolates (Table 1a). However, during the first 10 d of storage in the presence of potassium sorbate (ks) and under an ambient air (50% RH) environment, the *Penicillium* spp. (isolates API and MSI) exhibited a slower rate of visible growth than the *Aspergillus* sp. (isolate M4). Conversely, under high humidity with potassium sorbate (94% RH), all 3 mold spp. (API, M4, and MSI) exhibited a slower rate of visible growth during the first 10 d of storage (25°C) than the samples containing no potassium sorbate

Table 1b demonstrated the effectiveness of VOM as a food preservative. The only difference between Tables 1a and 1b is the presence of VOM in the headspace of the Scotchpak packaged samples of Table 1b (enrobed meat sandwiches).

Table 1a-Mold growth on shelf-stable sandwiches at 25 °C

 # positive/total # inoculated [no VOM]

	(RH: 50%)[a]						(RH: 94%)[b]					
Day	APT[c]		M4[d]		MSI[e]		APT		M4		MSI	
	-ks[f]	+ks	-ks	+ks	-ks	+ks	-ks	+ks	-ks	+ks	-ks	+ks
0	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
5	03	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
10	3/3	1/3	2/3	0/3	0/3	1/3	3/3	0/3	3/3	0/3	3/3	1/3
15	3/3	2/3	3/3	3/3	3/3	2/3	3/3	1/3	3/3	2/3	3/3	3/3
20	3/3	2/3	3/3	3/3	3/3	2/3	3/3	2/3	3/3	2/3	3/3	3/3
25	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3

[a] Storage equilibrum relative humidity (%) inside desiccator jar.

[b] Rel. humidity of storage environ. was adjusted to 94% with a saturated slurry of potassium nitrate.

[c,d,e] Mold spores used to inoculate shelf stable sandwiches(see Materials and Methods section).

[f] Neg. (-) and pos. (+) signs before ks denote the absence or presence of potassium sorbate.

Table 1b-Ability of VOM to control mold growth on shelf-stable sandwiches at 25°C.

	# Positive/total # inoculated [+ VOM]											
	(RH: 50%)[a]						(RH: 94%)[b]					
Day	APT[c]		M4[d]		MSI[e]		APT		M4		MSI	
	-ks	+ks	-ks	+ks	-ks	+ks	-ks	+ks	-ks	+ks	-ks	+ks
0	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
60	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
120	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3

[a] Storage equilibrum relative humidity(%) inside desiccator jar.

[b] Rel. humidity of storage environ. was adjusted to 94% with a saturated slurry of potassium nitrite.

[c,d,e] Mold spores used to inoculate shelf stable sandwiches (see M & M section).

[f] Neg. (-) and pos. (+) signs before ks denote the absence or presence of

Sandwiches that were stored with VOM labels remained free of visible mold growth for more than 100 days at 25°C (Table 1b). During the study, the levels of VOM within the desiccators were monitored (Fig. 3). The purpose for measuring gaseous VOM in the closed containers was to evaluate the effect of relative humidity on VOM vapors. Although the results (Fig. 3) indicated a 44 and 57% decrease in initial VOM concentrations in the headspace of both the low, and high moisture environments, VOM concentrations did remain relatively high in both environments after 120 d of storage at 25°C (200 - 300 ppm, Fig. 3). The unusually high headspace VOM concentration in the closed glass containers (desiccators) was due to the number of pouches (16/desiccator) each contained. Each of the 16 pouches contained four 40 mm x 40 mm VOM labels; thus the total number of VOM labels/desiccator was 24. Since these food products (sandwiches) were stored in closed glass containers, the decrease in VOM concentration during storage was most likely due to product uptake, since the RH did not appear to be a factor in the diminution of VOM. Studies have shown that foods packaged with VOM tend to retain a residual VOM odor when opened (Isshiki et al., 1992; Lin et al., 2000). However, the odor intensity and residual VOM levels can be controlled by selecting concentrations that effectively control the microbial population, but minimizes the residual odors (Isshiki et al., 1992; Lin et al., 2000).

The inclusion in this study of low and high Relative Humidity environments was to simulate possible field conditions that might be encountered during a military combat situation. Military rations [e.g., meal, ready-to-eat (MRE)] are packaged in gas impermeable tri-laminated film, and are designed to endure a minimum storage period of 3 years at 80° F, 6 months at 100°F, or 4 weeks at 120°F. Ordinarily, MREs would be susceptible to environmental stresses, such as temperature and humidity fluctuations; however, there are scenarios where military operations occur in remote theaters (tropical or arid environments).

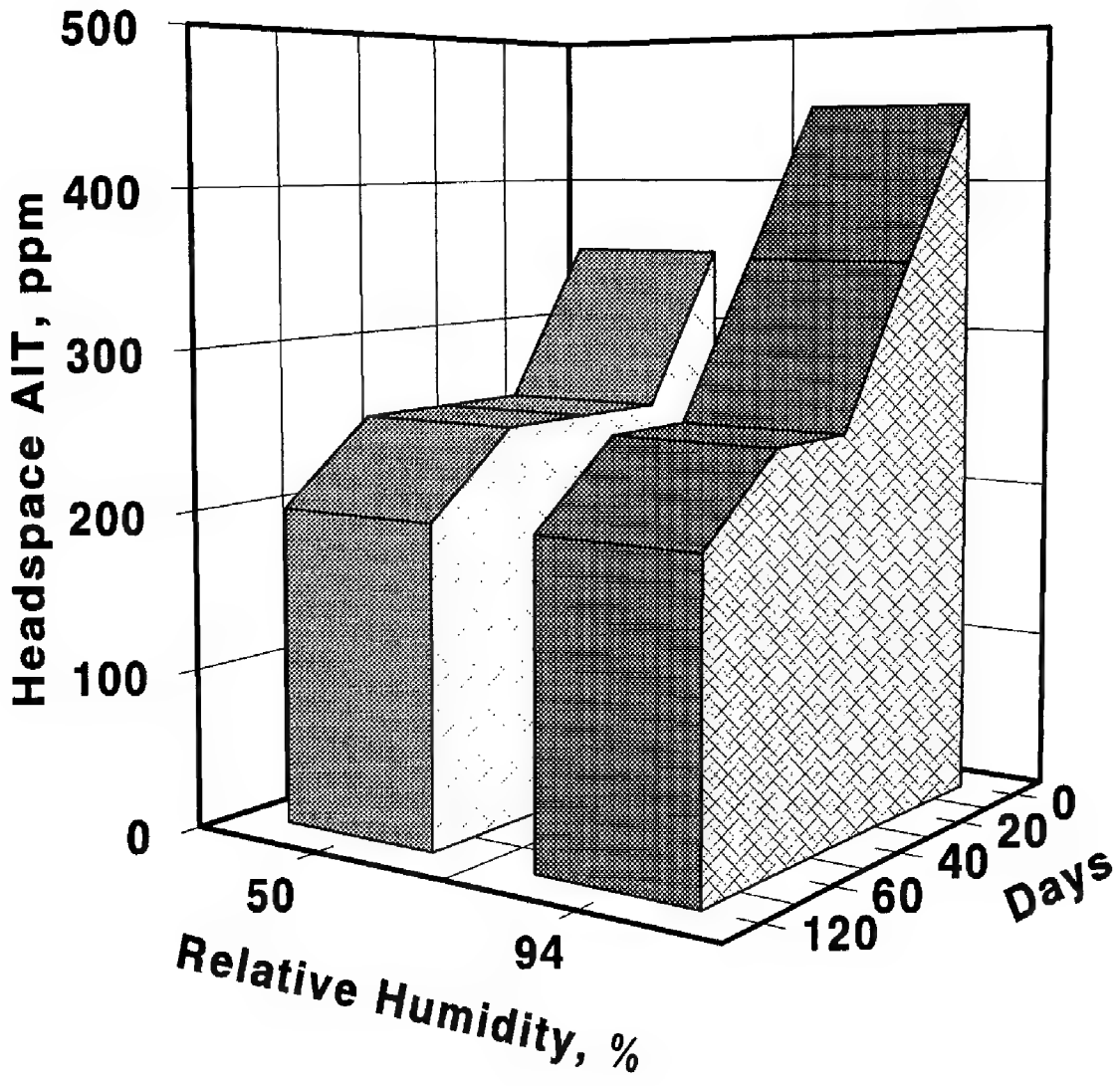


Figure 3. Changes in the headspace VOM concentration during storage (25°C) of beefstick sandwiches for 120 days under different relative humidities, 50 and 94 %.

Such military operations occasionally require a reliance on local indigenous food supplies. Under these circumstances, preservatives, such as VOM, become important factors, if extended military operations are required.

Control of yeast and mold growth on cheddar cheese packaged in gas permeable film with VOM

Cheddar cheese samples that were inoculated (yeast and mold cocktail), treated with two or four (40 mm x 40 mm VOM labels, packaged in heat sealable polyester film, and stored at 25°C for 21 d, resulted in an initial increase in colony forming units (Fig. 4b). For example, between 0 and 7 d, the yeast/mold counts in the control, T-I, and T-II, increased from approximately 1×10^4 YMC/g, to 4×10^6, 3×10^6, and 5×10^5 YMC/g, respectively (Fig.4b). At 7 d of incubation (25°C), there was no statistical difference ($P > 0.05$) between the yeast/mold counts in the control and T-1 (Fig. 4b), whereas treatment T-II (log 5.41) was significantly different from the control (log 6.64) and T-I (log 6.49, $P < 0.05$; Fig. 4b). During the same time interval (0 – 7 d), the headspace VOM concentration of T-I and T-II decreased ca. 20 to 23% from an initial level of 19 ppm (Fig. 4a). At the end of the 21 d incubation period, the headspace VOM concentration in both treatments had decreased to more than 50% of its initial concentration. Due to the fatty nature of the product (Cheddar cheese), the decrease in VOM during storage can reasonable be assigned to product uptake (bipolar compound, Lin et al., 2000) and diffusion through the packaging films. Both the inner (stomacher bag) and outer (Scotchpak pouches) wraps of the cheese product were gas permeable; therefore, VOM diffused across both films (stomacher bag and Scotchpak) at a rate that would be slower than if only one film was present. However, the slow release of VOM gas from the pouches resulted in a much diluted gaseous (VOM) environment inside the pouches. As a result, inhibition of growth of the fungi was marginal relative to the MRE packaging.

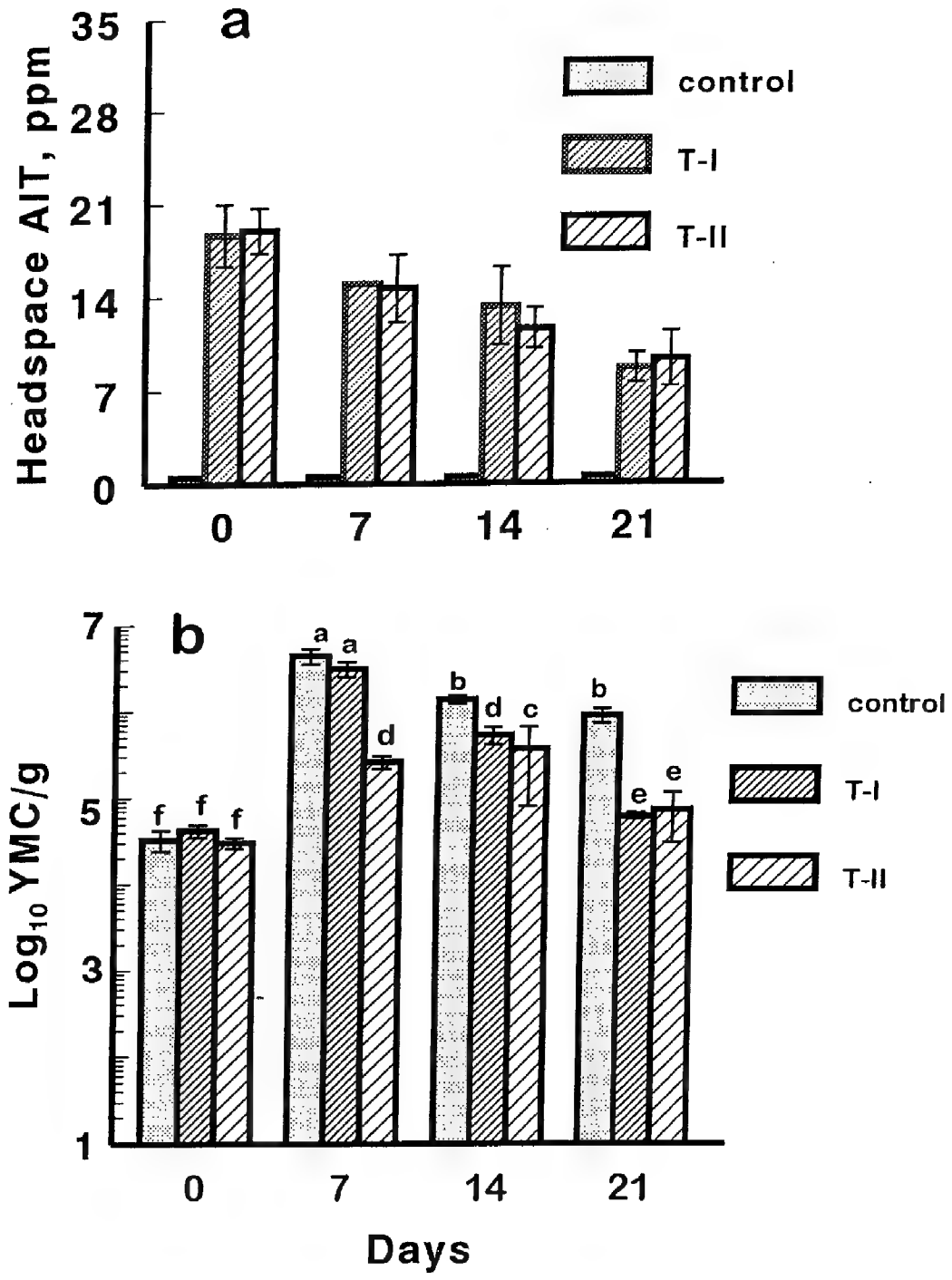


Figure 4. Effect of VOM vapor (a) on the growth of a yeast and mold cocktail (b) in shredded cheddar cheese stored for 21 days at 25°C. Cheese samples were placed in stomached bags and overwrapped with Scotchpak pouches. Treatments: control – no VOM labels; T-I, two 40 mm x 40 mm VOM labels; T-II, four 40 mm x 40 mm VOM labels. Prior to incubation, 120 cc of air was added to each Scotchpak pouch over wrap. Bars represent the mean of three replicates with two subsamples/replicate. Bars with the same superscript are not significantly different ($p > 0.05$).

Also, the permeability of the films permitted O_2 to diffuse into the pouches, which further negated the anti-mycotic effects of VOM. It was apparent from the results that the growth which occurred in the 0 to 7 d time interval was not impacted by the low concentration of VOM present in the headspace. At 14 days of incubation (25°C), a significant ($P < 0.05$), but small decrease ($\leq$ 1 log) occurred between treatments of the yeast/mold count (YMC/g) of the control and T-I sample, while there was no significant change in the YMC/g of treatment T-II, when compared to the 7 d count. Subsequently , the control did not change (21 d) significantly ($P > 0.05$) from the 14 d count; however, the two treatments, T-I (log 4.79) and T-II (log 4.86), differed significantly ($P < 0.05$) from their 14 day incubation counts (T-I = log 5.72 and T-II=log 5.57).

Control of yeast and mold growth on cheddar cheese packaged in gas impermeable pouches (MRE) with AIT

In a similar experiment in which cheddar cheese was packaged in gas impermeable MRE pouches, the results were quite different. Beyond day 0, no measurable (Fig. 5b) mold or yeast growth occurred in any of the inoculated test samples containing VOM (T-I, T-II and T-III). The initial (T-I = 15 ppm, T-II = 18 ppm, and T-III = 30 ppm), and final (T-I = 9.7 ppm, T-II = 10.7 ppm, and T-III = 12.8 ppm) headspace VOM concentrations inside the packages changed during the course of the 28 d storage period. In samples containing four (40 mm x 55 mm) AIT mats, T-III exhibited more VOM variability (range: 60 – 10 ppm) among packages, and the greatest decrease (Fig. 5a) in headspace VOM concentration among the three treatments: T-III (57% after 28 d), T-II (41%) and T-I (36%). The rationale for the large variations in T-III AIT concentrations is not clearly understood, but may be due to methodology (stain detector tubes) used

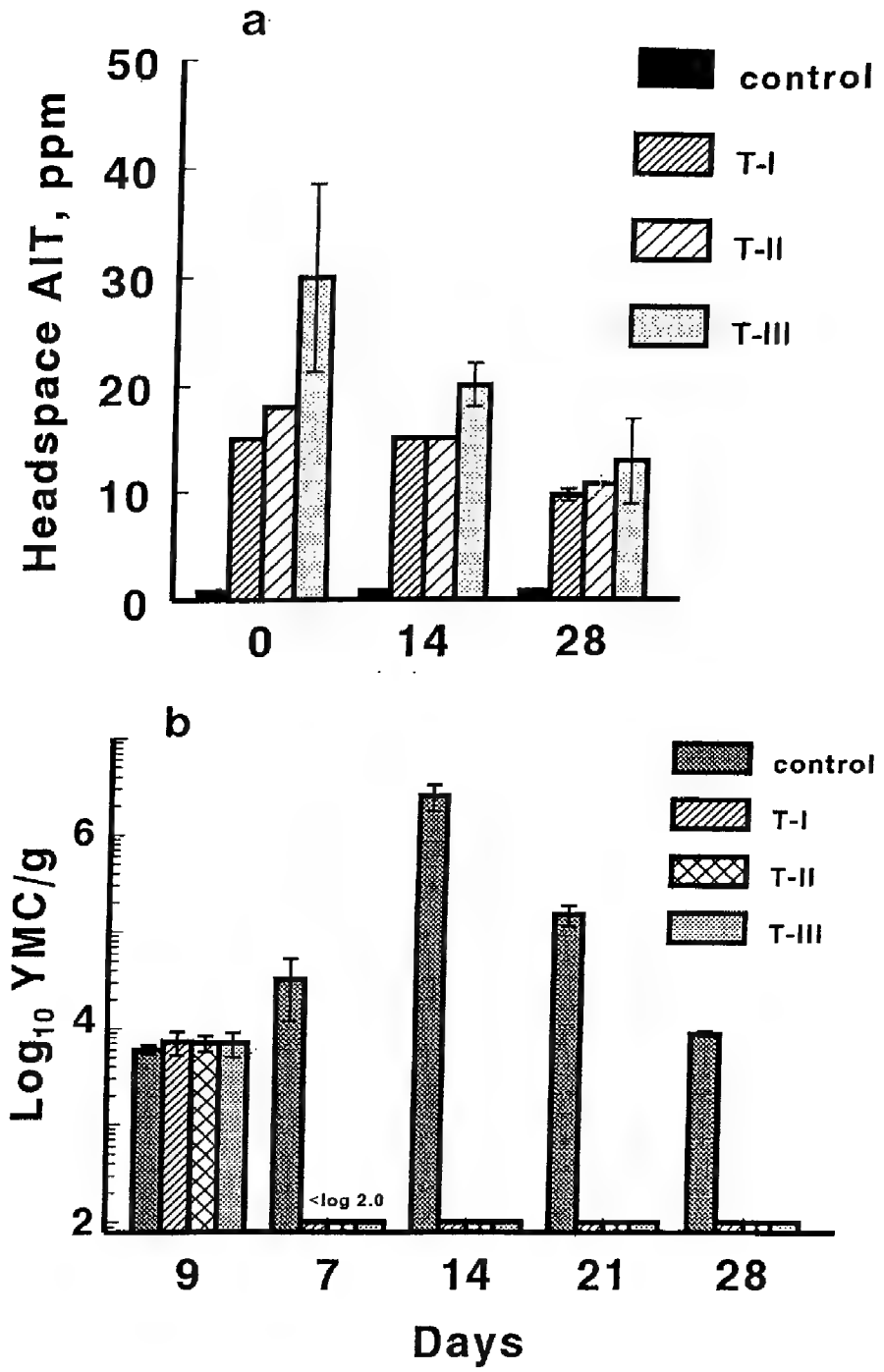


Figure 5. Effect of VOM vapor (a) on the yeast and mold cocktail (b) in shredded cheddar cheese packaged and stored for 28 days at 25°C. Cheese samples were placed in stomached bags and overwrapped with MRE pouches.Treatments: control – no VOM labels; T-I, two 40 mm x 40 mm VOM labels; T-II, four 40 mm x 40 mm VOM labels; T-III, four 40 mm x 55 mm VOM package mats. Prior to incubation, 120 cc of air was added to each MRE over wrap pouch. Bars represent the mean of three replicates with two subsamples/replicate.

to determine VOM concentrations in packages. However, the decrease in headspace AIT is believed to be solely due to product uptake. In a similar experiment conducted in our lab with pound cake slices packaged in MRE pouches with AIT, AIT was extracted from the cake crumb and analyzed by gas chromatographic methods. The results revealed product AIT uptake (unpublished). It is demonstrated from the results obtained in this study that cheese samples packaged in gas impermeable MRE pouches with AIT labels are less susceptible to the growth activities of the yeast/mold cocktail than the cheese packaged in the Scotchpak film (O_2 permeability: 5 cc/in^2/24 h).

The observed difference in fungal growth in the two packaging materials is might be attributed to the inability of the Scotchpak packaging film to retain a\ sufficient inhibitory level of AIT gas inside the pouches. Both the inner pouch (stomacher bag: O_2 transmission rate: 12,000 gm/m^2/24 hr) and the outer wrap (Scotchpak film), allowed a slow exchange of air (atmospheric) into and AIT out of the pouches. The AIT concentration inside the gas permable pouches was never static, but in a constant state of flux. This exchange eventually resulted in a decrease of effective inhibitory concentration of AIT; consequently, the yeast/mold population was less adversely impacted. Conversely, in MRE pouches, the AIT concentration inside the pouch after sealing remained unchanged (it was either absorbed or remained in the headspace above the food product) during storage, which resulted in effective inhibitory concentration of AIT against the yeast/mold population during the initial 7 d of storage (Fig. 5b).

Conclusions

Allyl isothiocyanate (VOM) vapor in concentrations > 200 ppm effectively eliminated the growth of three species of molds on an intermediate moisture shelf-stable sandwich for more than 120 d (Table 1). When cheddar cheese samples were packaged in gas impermeable MRE pouches ontaining 10 - 30 ppm VOM (headspace concentration), the yeast/mold growth activities were inhibited (Fig. 4a). Both shelf-stable sandwiches and cheese product were susceptible to VOM uptake. For this reason, compatible VOM levels must be established for each food product that will maximize the preservative effects and have a limited negative impact on sensory attributes. No sensory evaluation data was generated as part of this investigation; however, a good understanding of VOM absorption characteristics of the test products should allow selection and use of AIT levels demonstrated to control diverse microbial populations, yet minimize the impact of negative residual VOM odors in product. It was also demonstrated in this study that gas impermeable packaging (MRE) provided a superior barrier against VOM diffusion out of the packaging material, compared to the Scotchpak packaging material, which was unable to retain a sufficient inhibitory concentration of VOM. However, in order for VOM to be an effective preservative, adequate spacing must be provided inside the closed package so that all food surfaces are exposed to the gas. Intermediate Moisture Foods (IMF), e.g., MRE, maintain shelf stability at ambient temperature partially due to their low water activity (a_w), which does not favor the growth of most spoilage and pathogenic bacteria. However, due to non-uniform distribution of moisture in IMF, some mold and yeast will grow when the temperature and relative humidity provide them a favorable growth environment (Fontana, 2000). With the incorporation of VOM, either in the packaging sheet/mats or solution dipping, the undesirable growth of yeast and mold in IMF could be eliminated.

This document reports research undertaken at the U.S. Army Research, Development and Engineering Command, Natick Soldier Center, Natick, MA, and has been assigned No. NATICK/TR-05/007 in a series of reports approved for publication.

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